J. HYM. RES.
Vol. 10(2), 2001, pp. 163–180

Investigation of the Biology of Hymenoptera Associated with *Fergusonina* sp. (Diptera: Fergusoninidae), a Gall Fly of *Melaleuca quinquenervia*, Integrating Molecular Techniques

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Abstract.—The biologies of eleven species of Hymenoptera associated with the multi-locular galls of the fly, *Fergusonina* sp. (Fergusoninidae) were investigated. More than 2000 wasps were reared from 1100 galls collected in Queensland and New South Wales, Australia over a two-year period from 1997 to 1999 from *Melaleuca quinquenervia* (Myrtaceae). Additional galls from each site were dissected for observation and description of the immature stages. A molecular technique, which involved sequencing the D2 expansion domain of the 28S rRNA gene, was used to match the identity of the immature wasps with their adult forms. Of the eleven species of Hymenoptera associated with the *Fergusonina* sp. galls, we were able to observe and describe the biology of nine of the species. *Eurytoma* sp., *Coelocyba* sp, *Neanastatus* sp., *Cirrospilus* sp., *Bracon* sp., *Megastigmus* sp. and *Poecilocryptus nigromaculatus* Cameron, commonly or exclusively, fed directly upon the *Fergusonina* larvae and or pupae with most species developing on a single host. However, *Eurytoma* sp., *Bracon* sp., and *P. nigromaculatus* usually fed on multiple hosts. These species have specialized biologies, which enable them to chew through plant tissues to access gall inhabitants. *Chromeurytoma* sp. and *Euderus* sp. appeared to be hyperparasitoids based on the available evidence. The biological control implications of this suite of Hymenoptera are discussed in terms of their regulatory effect on *Fergusonina* sp., a potential biological control agent of *M. quinquenervia*, an invasive weed in Florida, USA.

Species of *Fergusonina* (Fergusoninidae) and their associated *Fergusobia* nematodes (Tylenchida: Sphaerulariidae), together form galls on the buds of their myrtaceous host plants (Currie 1937, Ferrar 1987, Giblin-Davis 2000). An undescribed species of *Fergusonina* and an undescribed *Fergusobia* form vegetative and floral galls on the broad-leafed paperbark tree, *Melaleuca quinquenervia* (Cavanilles) S.T. Blake (Fig 1). This *Fergusonina* sp. is present throughout the Australian distribution of *M. quinquenervia*, which stretches along the east coast from southern New South Wales (NSW), to the far north of Queensland (QLD). The gall-making cyclorrhaphous fly is under study as a potential biological control agent for *M. quinquenervia*, which was introduced from Australia into Florida in the United States in the early 1900's. In the last 30–40 years *M. quinquenervia* has greatly expanded its range in southern Florida, including the ecologically sensitive Everglades, where it now infests over 200,000 hectares causing extensive environmental and economic damage (Turner *et al.* 1998).

The seasonal phenology of *Fergusonina* sp. on *M. quinquenervia* was investigated by Goolsby *et al.* (2000a) over a two-year

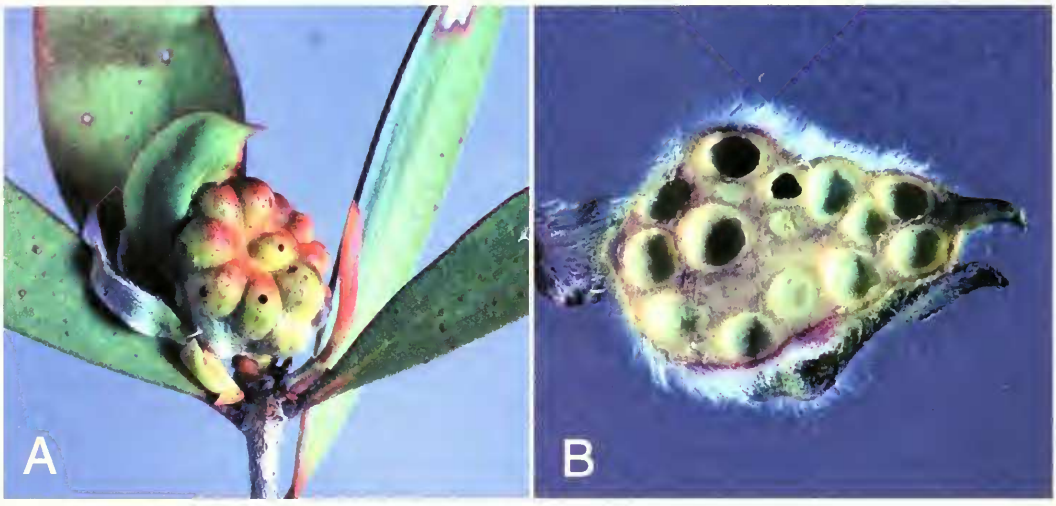

Fig 1. *Fergusonina* sp. gall on *Melaleuca quinquenervia*; a, Intact gall showing cluster of chambers, some with exit holes, and b, cross-section of gall showing individual chambers of the fly larvae.

period between 1997 and 1999. The study indicated that biotic factors, including parasitism, might have a significant effect on *Fergusonina* sp. gall density. Data collected for emergence of flies and associated gall inhabitants revealed numerous Hymenoptera, comprising nine species of Chalcidoidea and a single species each of Braconidae and Ichneumonidae, all potential parasitoids of the *Fergusonina* sp. In order to understand the impact of parasitism, we first needed to establish which species of wasps are primary parasitoids of the *Fergusonina* sp., which are hyperparasitoids or inquilines and which can behave as both primary parasitoids and hyperparasitoids. A large complement of primary parasitoid species may indicate that parasitism plays a significant role in regulating *Fergusonina* sp. populations in Australia. Understanding the regulatory effect of natural enemies on a potential biological control agent in its native range is a useful predictor of its success in its adventitious range.

In his pioneering work on the Fergusoninidae, Currie (1937) postulated that parasitic Hymenoptera played a major role in regulating their population dynamics. He reared many species of wasps from flower bud galls produced by *Fergusonina nicholsoni* Tonnoir on *Eucalyptus macrorhyncha* F. Mueller ex. Bentham and dissected galls to determine the biologies of their immatures. Currie noted that both a chalcidoid and a braconid were true parasitoids of the gall-making flies and briefly listed four other species of chalcidoid wasps that formed independent chambers within the galls. However, more detailed information on the biologies of the gall-associated wasps was never published. Taylor *et al.* (1996) reared twelve species of wasps from leaf galls formed by *Fergusonina flavicornis* Malloch on *Eucalyptus camaldulensis* Dehnhardt in South Australia. They did not dissect galls, but discussed the probable biologies of the various wasp species in the light of their relative abundance and the biologies of related species. Both studies found an abundance of gall-associated Hymenoptera, but were largely unable to positively determine their role inside the gall.

Gall-making agents interact with associated parasitoids, predators and inquilines behind the cover of plant tissue that often obscures our understanding of their biologies. Because it may be difficult to identify the hymenopteran larvae associ-

ated with galls, many studies fail to associate the biology of the immatures with their adult form (Shorthouse *et al.* 1990, Manongi and Hoffman 1995). The most common method for determining the biology of immatures is to observe them in the gall and then hold them until they emerge as adults, which can be more easily identified. This method is the most straightforward and has been used widely in the study of gall inhabiting Hymenoptera.

However, this method is time consuming and may not be practical when dealing with galls that contain a large suite of parasitoid species. In our study we also dissected and observed gall contents, but combined this method with a molecular technique which involved sequencing the D2 expansion domain of the 28S rRNA gene to match the identity of the wasp larvae with their adult forms. The D2 expansion domain of the 28S rRNA gene has been used in other studies to separate cryptic species of adult hymenopteran parasitoids (De Barro *et al.* 2000, Babcock and Heraty 2000), and aquatic weevils (Goolsby *et al.* 2000b). Tilmon *et al.* (2000) used the COI gene to determine species composition of immature parasitoids in their host. We used the molecular method of sequencing the D2 gene as a way to determine identity of the immatures as we observed their biology *in vivo*.

MATERIALS AND METHODS

Monthly collections of mature *Fergusonina* sp. galls on *M. quinquenervia* trees were made from Peregian and Morayfield (QLD) and Woodburn (NSW) from July 1997 to September 1999. The locations and phenology of the *Fergusonina* sp. are described in Goolsby *et al.* (2000a). Galls were held for one month in ventilated containers for emergence of the gall inhabitants. The emerged insects were counted and sorted to species.

In September 1999, following the two-year study, approximately 30 galls were collected from each site in order to observe and investigate the biology of the gall inhabitants. We dissected several hundred gall chambers in order to observe the behavior of the gall inhabitants. Observations of the gall insects were made using a dissecting microscope, and pictures of the contents were taken using a digital camera (Sony Mavica, model FD-88). Owing to the mobility of the camera, pictures of immatures could be taken by focusing through the ocular of the microscope. Immatures were placed in vials of 95% alcohol for DNA analysis. Several specimens of each species were analyzed. Adult parasitoids were identified to genus and, where possible, to species. Vouchers are located in the Queensland Museum, Brisbane; Florida State Collection, Gainesville and the U.S. National Museum, Washington, D.C.

Eggs, larvae, and pupae of Hymenoptera were used for gene sequencing. Gene sequences of the immature Hymenoptera were compared with adults that had been reared from *Fergusonina* sp. galls. Adult representatives of the less common Hymenoptera species were reared from *Fergusonina* sp. galls collected during the previous two years. We sequenced the D2 expansion domain of the 28S rRNA, which ranged from 564 to 593 base pairs long depending on the species. The methods were those described by De Barro *et al.* (2000).

The polymerase chain reaction (PCR) was used to amplify the D2 gene regions for each specimen. Primers for the region followed Campbell *et al.* (1993); D2F 5′-CGTGTTGCTTGATAGTGCAGC-3′ and D2R 5′-TTGGTCCGTGTTTCAAGACGG-3′, or ND2F 5′- AGTACCGTGAGGGAAAGTTG-3′, which was used in some reactions as an alternate forward primer which anneals approximately 90 bases down-stream of the D2F binding site. All reaction volumes were 50 μl, containing 20 pM of each primer, 200 μM each dGTP, dATP, dCTP and dTTP, 1.5–2.5 mM $MgCl_2$, 2 μl DNA lysate, 1X supplied buff-

Table 1. Gall insects reared during two-year field study.

Site: / Galls:	Peregian 366	Morayfield 493	Woodburn 263	All sites 1122
Fergusonina sp.	372	483	420	1275
Eurytoma sp.	300	473	13	786
Coelocyba sp.	276	33	122	431
Neanastatus sp.	65	144	35	244
Cirrospilus sp.	113	19	0	132
Bracon sp.	28	42	47	117
Eupelmus semiputata	4	99	0	103
Chromeurytoma sp.	15	50	5	93
Megastigmus sp.	17	35	28	80
Eupelmus (*Eupelmus*) sp.	3	5	2	10
Euderus sp.	3	4	2	9
Poecilocryptus nigromaculatus	0	1	4	5
Total Hymenoptera	824	905	258	2010
% Parasitism	68.90%	65.20%	38.05%	61.19%

er and 2.5 U Taq polymerase (Bresatec, Australia). PCR amplification was done using a Hybaid thermocycler using the following parameters. A pre-cycle denaturation step for 5 min at 94°C, followed by the addition of the Taq polymerase. Then, 35 cycles of 1 min at 94°C, 1 min at 55°C and 1.5 min at 72°C followed by a final post-cycle extension step at 72°C.

The D2 amplicons were purified and prepared for sequencing by electrophoresis in 0.8% TAE agarose gels containing 10 μg ml^{-1} ethidium bromide (Sambrook *et al.* 1989). Fragments were excised and transferred to a microfuge tube. The agarose slices were mashed in 30 μl sterile distilled water using a toothpick, then incubated at 50°C for 1 h. Samples were left at room temperature overnight to allow the DNA to elute from the gel. The samples were stored at −20°C until required.

Five microliters of the eluted PCR-amplicons and the appropriate PCR-primers were used for sequencing according to the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit Manual (PE-Applied BioSystems). Both strands of each fragment were sequenced and reactions were loaded onto an Perkin Elmer, Applied Biosystems Division, Model 377 ABI PRISM Genetic Analyzer. All sequences were deposited in GenBank® (see Table 2).

RESULTS AND DISCUSSION

Eleven species of Hymenoptera were reared from the *Fergusonina* sp. galls over the two-year sampling period (Table 1). *Eurytoma* sp. was the most common species at Peregian and Morayfield, whereas *Coelocyba* sp. was most common at Woodburn. *Eurytoma* sp. and *Coelocyba* sp. comprised 61% of the 2010 specimens of Hymenoptera reared from the galls over the two-year period. Parasitism of the *Fergusonina* sp. larvae and pupae was high (> 60%) at both Morayfield and Peregian. The pooled percentage parasitism for the three sites over the two year period was 61.2% (Table 1).

We identified the immatures of eight of the eleven species collected in the study by matching their DNA sequences with those of the adult forms (Table 2). The larvae of a ninth species were identified by examining larval exuviae recovered from *Fergusonina* puparia from which parasitoids had emerged. Immatures of the remaining two species were not encountered; their biology was deduced from published information of congeners. All of the immatures analyzed were matched with adult forms except for one hyperparasitoid egg. The D2 sequence from the hyperparasitoid egg was unique and not de-

Table 2. Gall insects identified in study using D2 sequence data.

	Species*	Location	Stage	GenBank accession number	Selection criteria
1	*Fergusonina*–Male	MORAYFIELD	Adult	AF345569	Primary gall former
2	*Fergusonina*–Female	WOODBURN	Adult	AF345570	Primary gall former
3	*Fergusonina*	MORAYFIELD	Larva	AF345571	Primary gall former
4	*Eurytoma*	MORAYFIELD	Adult	AF345572	Reared from individual that had predated on multiple hosts
5	*Eurytoma*	PEREGIAN	Adult	AF345573	Reared from individual that had predated on multiple hosts
6	*Eurytoma*	PEREGIAN	Adult	AF345612	Reared from pupa in chamber with copious masticated gall tissue and black meconium
7	*Eurytoma*	MORAYFIELD	Larva	AF345574	Larva had fed on multiple hosts, chamber contained ball of *Fergusonina* remains
8	*Eurytoma*	PEREGIAN	Larva	AF345575	Larva had fed on multiple hosts, copious amounts of masticated gall tissue present
9	*Eurytoma*	MORAYFIELD	Larva	AF345613	Larva collected at center of young gall with connections to two other chambers
10	*Eurytoma*	MORAYFIELD	Larva	AF345606	Larval remains being consumed by *Bracon* larva
11	*Eurytoma*	PEREGIAN	Larva	AF345610	Larva with *Poecilocryptus* larva attached (Specimen 53)
12	*Eurytoma*	MORAYFIELD	Larva	AF345607	*Fergusonina* parasitoid in clean gall chamber, attacked by hyper-parasitoid below (Specimen 13)
13	Hyperparasitoid egg	MORAYFIELD	Egg	AF345608	Egg attached to *Eurytoma* larva (Specimen 12 above)
14	*Coelocyba*	PEREGIAN	Adult	AF345576	Reared from naked pupa, chamber contained *Fergusonina* remains
15	*Coelocyba*	PEREGIAN	Adult	AF345577	Reared from an intact *Fergusonina* puparium
16	*Coelocyba*	PEREGIAN	Adult	AF345614	Reared from an intact *Fergusonina* puparium
17	*Coelocyba*	PEREGIAN	Adult	AF359455	Reared from an intact *Fergusonina* puparium
18	*Coelocyba*	WOODBURN	Larva	AF359454	Larva restricted to single chamber
19	*Neanastatus*	MORAYFIELD	Adult	AF345580	Reared from *Fergusonina* gall
20	*Neanastatus*	PEREGIAN	Adult	AF345582	Reared from *Fergusonina* gall
21	*Neanastatus*	MORAYFIELD	Pupa	AF345581	Naked pupa restricted to single chamber
22	*Neanastatus*	PEREGIAN	Larva	AF345583	Larva collected in single chamber, cradling remains of *Fergusonina*
23	*Neanastatus*	MORAYFIELD	Larva	AF345584	Same as above, but from different site
24	*Neanastatus*	MORAYFIELD	Larva	AF345615	Larva collected from single isolated chamber
25	*Cirrospilus*	PEREGIAN	Adult	AF345585	Reared from *Fergusonina* gall
26	*Cirrospilus*	MORAYFIELD	Adult	AF345586	Reared from *Fergusonina* gall
27	*Cirrospilus*	MORAYFIELD	Adult	AF345616	Reared from *Fergusonina* gall
28	*Cirrospilus*	PEREGIAN	Larva	AF345604	Collected from isolated chamber with remnants of *Fergusonina*
29	*Cirrospilus*	PEREGIAN	Larva	AF345605	Collected from isolated chamber with remnants of *Fergusonina*
30	*Bracon*	WOODBURN	Adult	AF345587	Reared from silk cocoon inside *Fergusonina* gall

Table 2. Continued.

	Species*	Location	Stage	GenBank* accession number	Selection criteria
31	*Bracon*	MORAYFIELD	Adult	AF345588	Reared from *Fergusonina* gall
32	*Bracon*	MORAYFIELD	Larva	AF345589	Larva collected as it was making silk cocoon
33	*Bracon*	MORAYFIELD	Larva	AF345590	Larva collected as it straddled two chambers, both full of copious masticated gall tissue and *Fergusonina* remains.
34	*Bracon*	MORAYFIELD	Larva	AF345617	Larva collected from gall with interconnected chambers, full of masticated gall tissue and *Fergusonina* remains
35	*Eupelmus semiputata*	MORAYFIELD	Adult	AF345591	Reared from *Fergusonina* gall
36	*Eupelmus semiputata*	MORAYFIELD	Adult	AF345592	Reared from *Fergusonina* gall
37	*Eupelmus semiputata*	MORAYFIELD	Adult	AF345618	Reared from *Fergusonina* gall
38	*Chromeurytoma*	PEREGIAN	Adult	AF345595	Found in single isolated gall chamber
39	*Chromeurytoma*	MORAYFIELD	Adult	AF345596	Reared from *Fergusonina* gall
40	*Chromeurytoma*	MORAYFIELD	Adult	AF345620	Reared from *Fergusonina* gall
41	*Chromeurytoma*	MORAYFIELD	Larva	AF345597	Collected from chamber with *Neanastatus* pupal remains
42	*Chromeurytoma*	MORAYFIELD	Larva	AF345598	Collected from chamber with *Neanastatus* pupal remains
43	*Chromeurytoma*	MORAYFIELD	Larva	AF345593	Larva collected from intact chamber with wasp pupal remains
44	*Megastigmus*	MORAYFIELD	Adult	AF345594	Reared from *Fergusonina* pupal case
45	*Megastigmus*	WOODBURN	Adult	AF345619	Reared from *Fergusonina* gall
46	*Eupelmus* (*Eupelmus*)	MORAYFIELD	Adult	AF345599	Reared from *Fergusonina* gall
47	*Eupelmus* (*Eupelmus*)	PEREGIAN	Adult	AF345600	Reared from *Fergusonina* gall
48	*Eupelmus* (*Eupelmus*)	PEREGIAN	Adult	AF345621	Reared from *Fergusonina* gall
49	*Euderus*	PEREGIAN	Adult	AF345601	Reared from *Fergusonina* gall
50	*Euderus*	WOODBURN	Adult	AF345602	Reared from *Fergusonina* gall
51	*Euderus*	MORAYFIELD	Egg	AF345609	Egg collected from deflated, dead *Poecilocryptus* larva
52	*Poecilocryptus nigromaculatus*	WOODBURN	Adult	AF345603	Adult collected from *Fergusonina* gall
53	*Poecilocryptus nigromaculatus*	PEREGIAN	Larva	AF345611	Hyperparasitoid of *Eurytoma* larva (Specimen 11)

* No variation was noted in D2 gene sequence data between individuals of each taxon.

tected again. Most species develop in a single intact gall chamber as primary parasitoids of *Fergusonina* sp. The larvae of three species, *Eurytoma* sp., *Bracon* sp. and *Poecilocryptus nigromaculatus* tunnelled between gall chambers to feed on multiple hosts. Some authors (eg. Godfray 1994) prefer the term predator to describe this biology. We consider the distinction between predator and parasitoid to be ambiguous and prefer to describe these species as parasitoids feeding on multiple hosts. Several hyperparasitoid species were identified either by their eggs, that were found attached to parasitoid larvae, or by their larvae that occupied chambers containing the remains of parasitoid larvae or pupae.

Biology of Associated Hymenoptera

Eurytoma sp. (Eurytomidae).—Specimens of *Eurytoma* emerged from galls from all three sites and made up 39.1% of the total number of Hymenoptera reared from the field collections. They were the most numerous gall-associated wasps at

the two Queensland localities, Peregian and Morayfield. Few *Eurytoma* were recovered from galls collected at Woodburn (Table 1). Despite some variation in the coloration of their legs, all specimens appear to belong to a single species. The D2 gene sequences of adults from Peregian and Morayfield were identical (Table 2).

Eurytoma is an enormous cosmopolitan genus; Bouček (1988) listed 66 species from Australia and indicated there were many still undescribed. In the absence of revisionary studies on Australian species of *Eurytoma* it was not possible to identify this species. However, it appears to be allied to a distinctive group of Australian *Eurytoma* discussed by Bouček (1988) and characterized mainly by an elongate petiole and relatively long marginal vein. This group includes *E. longipetiolata* Girault and *E. australiensis* Ashmead (Bouček 1988) and after examination of their types by CJB, we believe that *E. carlylei* Girault and *E. herbertensis* Girault probably also belong here. The species of *Eurytoma* reared from *Fergusonina* galls possesses several characters of the group including an elongate female petiole that is longer than the hind coxae and slightly curved, and a laterally compressed gaster that has the combined length of the first three gastral tergites less than their height and shorter than the length of the fourth gastral tergite (Bouček 1988). However, species of the group have the marginal vein 2.5–3× longer than the stigmal vein (Bouček 1988) while the *Eurytoma* sp. from the galls has the marginal vein only about twice the length of the stigmal.

Eurytoma sp. larvae had a relatively large head capsule with the mouthparts directed ventrally. The mandibles of mature larvae were bidentate with a strong, acute, apical tooth and an acute subapical tooth about half the length of the apical. Larvae had a series of conspicuous dorsal protuberances on the meso- and metathorax and the first eight abdominal segments. Larvae were only moderately setose with most thoracic and abdominal setae short and inconspicuous. However, each thoracic segment had three pairs of longer setae ventrad of the spiracles.

The larval biology of *Eurytoma* sp. was variable. Commonly larvae fed on multiple *Fergusonina* larvae and possibly the larvae of other gall-associated Hymenoptera. Occasionally individual *Eurytoma* pupae were observed in single intact chambers, having completed their development on only one *Fergusonina* host. Molecular data confirmed that *Eurytoma* developing on single or multiple hosts were the same species.

Eurytoma sp. larvae feeding on multiple hosts were found in chambers that were connected by small holes to one or more other chambers. These chambers were typically filled with brown, particulate debris that we interpreted as masticated, but not ingested, gall tissue. In addition, dissociated plates from the dorsal shields of *Fergusonina* larvae were found amongst the brown debris. Frequently several *Eurytoma* larvae completed development in a single gall.

Eurytoma is a diverse genus with a wide array of larval biologies ranging from entomophagous to phytophagous species. Many species attack gall formers (Di Guilio 1997) including some that feed on several hosts in multi-chambered galls (Blair 1944, Bouček 1988), which is similar to the species in our study. The larvae of some gall-inhabiting species of *Eurytoma* have been reported to feed on both insect and plant tissue (Varley 1937, Noble 1941, Askew 1961). Although the *Eurytoma* larvae in our study masticated gall tissue, it is not clear if they derived any nutritional benefit from this activity, or it they just mechanically scraped away the tissue to gain access to additional chambers.

All studies on the Hymenoptera associated with *Fergusonina* galls have recorded *Eurytoma* species (Currie 1937, Harris 1982, Taylor *et al.* 1996) but only Currie investigated the larval biology. Unlike our

study, he found that larvae of *Eurytoma* "varirufipes" (an unpublished Girault manuscript name), were inquilines within galls of *Fergusonina nicholsoni* Tonnoir on *Eucalyptus macrorhyncha*, forming separate chambers to those of the fly larvae.

Coelocyba sp. (Pteromalidae).—Overall, *Coelocyba* sp. was the second most abundant wasp reared (21.4% of total specimens), but was uncommon at the Morayfield site (Table 1). Bouček (1988) listed nine species of the endemic Australian genus *Coelocyba*, which he noted was composed of two species groups separated on the structure of the dorsellum and propodeum. The species reared from *Fergusonina* sp. galls belongs to the group containing *C. nigrocincta* Ashmead, characterized by the posterior margin of the dorsellum being broadly rounded (Bouček 1988). *Coelocyba* sp. closely resembles *C. nigrocincta* in color pattern, however species in the genus are difficult to recognize and the value of color in distinguishing species is questionable (Bouček 1988). The genus is in need of revision (Bouček 1988) and consequently precise identification of the species reared in the study was not possible.

Coelocyba larvae appear almost glabrous, distinguishing them from most larvae encountered during dissections, except those of *Cirrospilus*. *Coelocyba* larvae can be distinguished from *Cirrospilus* larvae by their globular head capsules and ventrally directed mouthparts. In addition, *Coelocyba* larvae have tridentate mandibles with a strong, acute apical tooth; a closely appressed, acute, subapical tooth; and a small basal tooth.

Adults of *Coelocyba* sp. emerged either from naked wasp pupae or from intact *Fergusonina* puparia in approximately equal proportions. The D2 gene sequences of adults reared from both were identical (Table 2). *Coelocyba* that emerged from *Fergusonina* puparia either developed as true endoparasitoids or more probably as ectoparasitoids of the pharate *Fergusonina* pupa. Chambers that contained parasitized *Fergusonina* puparia closely resembled those with unparasitized puparia. Parasitized puparia were attached to the wall of the chamber by the normal transparent, elastic substance (see Currie 1937: 150). Naked pupae and larvae of *Coelocyba* were always found singly in isolated chambers, along with pale granules of host remains containing *Fergusonina* dorsal plates. In these cases *Coelocyba* developed as a primary ectoparasitoid of the *Fergusonina*. We found no evidence that *Coelocyba* larvae fed on gall tissue. Chambers with *Coelocyba* pupae also contained a patch of dark meconium.

Known species of *Coelocyba* are associated with gall-inducing pteromalids and fergusoninids (Bouček 1988), but their precise larval biologies are unknown. Bouček (1988) and Taylor *et al.* (1996) reported that larvae of *C. nigrocincta* Ashmead had been demonstrated to be inquilines in the galls of *Perilampella hecataeus* (Walker) on *Acacia decurrens* Willdenow, primarily based on work done by Noble (1941). They claimed that the *C. nigrocincta* larva killed the resident gall-inducer and formed its own cell and fed on the gall tissue. However, although Noble (1941) reported that the *C. nigrocincta* larva killed the larva of the gall-inducer, he made no mention of it forming its own chambers, or of it feeding on gall tissue. In our study we found no evidence that *Coelocyba* sp. larvae fed on gall tissue and concluded that they were almost always primary parasitoids of the *Fergusonina* larvae or pupae. This is in agreement with Currie (1937) who briefly noted that the species of *Coelocyba* that he reared from flower bud galls produced by *Fergusonina nicholsoni* on *Eucalyptus macrorhyncha* was a "true parasite" of the fly larvae. Taylor *et al.* (1996) reared *C. nigrocincta* from leaf bud galls on *E. macrorhyncha* but did not investigate its larval biology.

Neanastatus sp. (Eupelmidae).—A single species of *Neanastatus* was moderately

common at all three sites (Table 1) and accounted for 12.1% of the total specimens reared. Molecular sequences of adults from Morayfield and Peregian were identical (Table 2). Species of *Neanastatus* are apparently restricted to the Old World (Gibson 1989), but are widely distributed from southern Europe through Africa, and southern Asia to Australia with most species known from the tropics (Bouček 1988). Bouček (1988) lists 21 Australian species, all described by A. A. Girault. Specimens reared from galls of *Fergusonina* sp. on *M. quinquenervia* have the head and most of the meso- and metasoma dark-colored, mostly with metallic green reflections. At least the anterior half of the pronotum and most of the first gastral tergite is yellowish. The hind tibia is mostly black with a narrow, basal, white band. Amongst the Australian species, they most closely resemble *N. flavipronotum* Girault. However the holotype of this species differs in that the lower face surrounding the mouthparts is yellowish. In addition, the pronotum is extensively yellowish with only a narrow posterior dark band.

Neanastatus sp. larvae are whitish and fusiform, gradually tapering posteriorly. The larval mandibles are simple, each with a single acute tooth. *Neanastatus* larvae are conspicuously setose, with rows of long lateral setae on the thoracic and abdominal segments, except the first abdominal segment. The thoracic segments have two additional pairs of long setae. The larvae can be distinguished from the setose larvae of *Chromeurytoma* (see below) by the absence of ventrolateral setae on the abdominal segments. *Neanastatus* sp. pupae can be distinguished by a conspicuous tubercle on the dorsal frons.

The available evidence indicates that *Neanastatus* sp. develops as a solitary, primary ectoparasitoid of *Fergusonina* larvae. In all instances, *Neanastatus* larvae, pupae and newly eclosed adults were found singly inside isolated, intact chambers. Mature larvae were observed resting on their dorsal surfaces and cradling, on their ventral surfaces, small balls of tissue containing *Fergusonina* dorsal plates. There was no indication of *Neanastatus* larvae feeding on gall tissue. *Neanastatus* pupae occupy relatively clean chambers that contain a dark patch of tar-like larval meconium; one or sometimes two shriveled, setose, larval exuvia; and usually the remains of a *Fergusonina* larva indicated by the presence of fragments of the dorsal shield.

Species of *Neanastatus* have been recorded as parasitoids in the galls of cecidomyiid flies, especially those associated with grasses and herbaceous plants (Bouček 1988). According to Gibson (1989) they have either been recorded as primary parasitoids of the fly larvae or as hyperparasitoids through Platygasteridae (Hymenoptera: Platygasteroidea). The biologies of Australian *Neanastatus* are largely unknown, although one species has been reared from galls on *Eremocitrus* (Rutaceae) (Naumann 1991) and CJB has seen a specimen reared from an unidentified gall on *Brachychiton discolor* F. Mueller (Sterculiaceae). One Australian species, *Neanastatus cinctiventris* Girault, is known to be a parasitoid of the Rice gall-midge, *Orseolia oryzae* (Wood-Mason), throughout southeast Asia. This is the first record of a species of *Neanastatus* attacking a fergusoninid fly. Interestingly, *Neanastatus* has not been reared from several hundred cecidomyiid galls collected from *Melaleuca quinquenervia* (unpublished data).

Cirrospilus sp. (Eulophidae).—A single species of *Cirrospilus* was moderately common at Peregian where it was the third most numerous species emerging from galls (Table 1). However, *Cirrospilus* sp. was rare at Morayfield and was not recovered from galls at Woodburn. Molecular sequences of adults from Morayfield and Peregian were identical (Table 2). In total, this species comprised 5.6% of the specimens reared. *Cirrospilus* is a large, morphologically diverse, cosmopolitan genus

with something in the order of 60 described species from Australia (Bouček 1988). In the absence of any revisionary work on Australian *Cirrospilus*, it was not possible to identify the species from *M. quinquenervia* galls. However, it belongs to a group of species that roughly corresponds to A.A. Girault's genus *Gyrolasella* that was synonymised with *Cirrospilus* by Bouček (1988). The color pattern of the species reared from *Fergusonina* sp. galls was similar to that of a number of Australian *Cirrospilus* species that have the body mostly yellowish with metallic green markings on the head and mesosoma and a series of dark brown or black transverse bands on the gaster. The species in our study was similar to the *Cirrospilus* reared from *Fergusonina flavicornis* Malloch galls by Taylor *et al.* (1996, Fig. 14) but had less extensive metallic green on the occiput and the median lobe of the mesoscutum.

Mature larvae of the *Cirrospilus* sp. reared in this study were distinctive and easily distinguished from those of other wasps associated with the galls. The larval head capsule was virtually prognathous, dorsoventrally flattened and with broad, cheek-like, lateral expansions basally. The mandibles were sickle-shaped and unidentate. The head, thorax and abdomen appeared more or less glabrous, without any conspicuous setae. The thorax and abdomen had three and eight low, dorsal protuberances respectively.

The available evidence indicated that *Cirrospilus* sp. developed as a solitary, primary ectoparasitoid of third instar Fergusonina larvae. In all instances, *Cirrospilus* larvae and pupae were found singly, inside isolated, intact chambers. Chambers with larvae usually also contained pale granules of host remains and *Fergusonina* dorsal plates. We also observed intact but shrivelled third instar *Fergusonina* larvae together with *Cirrospilus* larvae. We found no evidence of *Cirrospilus* larvae feeding on gall tissue or acting as hyperparasitoids. On first inspection, chambers with *Cirrospilus* pupae usually appear empty of host remains but contain a thick patch of meconium. On closer inspection, plates from *Fergusonina* dorsal shields were nearly always incorporated into the patch of meconium.

Cirrospilus is a biologically diverse genus with species developing as parasitoids or hyperparasitoids, commonly of leafminers, or of other larvae and pupae in concealed situations (Bouček 1988). In Australia, many species are associated with leaf galls, especially on eucalypts (Bouček 1988). Taylor *et al.* (1996) reared a species of *Cirrospilus* from leaf-galls of *Fergusonina flavicornis* on *Eucalyptus camaldulensis*.

Bracon sp. (Braconidae).—A single species of *Bracon* was recovered in relatively low numbers from all the sites comprising 5.8 % of the specimens reared, but it was the second most common species at Woodburn (Table 1). Molecular sequences of adults from Woodburn and Peregian were identical (Table 2). *Bracon* is a very large, cosmopolitan genus with many Australian species, most of them undescribed (Austin and Faulds 1989, Quicke and Ingram 1993).

Mature larvae of *Bracon* sp. were distinguished from those of most other wasps associated with the galls, except *Poecilocryptus nigromaculatus* (see below), by their large size. They were also characterized by distinctive labial and maxillary sclerites, and large, heavily sclerotized, unidentate mandibles, which had a series of serrations on their inner margins.

Typically *Bracon* larvae fed indiscriminately on hosts within the galls, entering multiple chambers and consuming a succession of *Fergusonina* larvae and the larvae and pupae of the other wasps associated with the galls. Often two or more *Bracon* larvae completed development within the same gall. Galls occupied by mature *Bracon* larvae usually had several chambers interconnected by relatively large holes. The chambers were generally

packed with brown, particulate debris that we concluded was masticated gall tissue. Chambers also frequently contained dissociated *Fergusonina* dorsal-shield plates. The remains of a *Neanastatus* pupa and a small *Poecilocryptus nigromaculatus* larva were also found within chambers occupied by *Bracon* larvae. On one occasion a *Bracon* larva was directly observed feeding on a *Eurytoma* larva (Table 2). This is the first record of a species of *Bracon* acting as a facultative hyperparasitoid. Other known species of the genus are primary ectoparasitoids (Shaw and Huddleston 1991). Pupation occured in a relatively loosely woven silk cocoon with brown debris incorporated on its outer surface. The cocoon usually filled two gall chambers and had a mass of dark meconium deposited at one end.

Species of *Bracon* attack diverse hosts but many are parasites of concealed larvae, mostly of Lepidoptera but also Coleoptera and Hymenoptera-Symphyta (Quicke and Ingram 1993). Several species also parasitize Diptera, particularly gall-making larvae (Quicke and Sharkey 1989). This is the second record of a species of *Bracon* from a fergusoninid gall, Taylor *et al.* (1996) having reared *B. fergusoninus* Taylor, Austin and Davies from *Fergusonina flavicornis* leaf-galls on *Eucalyptus camaldulensis*. Currie (1937) also reared an unidentified braconid from *F. nicholsoni* flower-bud galls on *E. macrorhyncha*. He reported that the braconid larvae feed "indiscriminately on gall tissues and fly larvae" and it seems likely that their biology is similar to the *Bracon* sp. in our study. However, although we confirm that the *Bracon* larvae masticate a considerable amount of gall tissue, evidenced by copious amounts of brown debris, it is unclear whether they derive nutrition from this activity or just mechanically scrape away the tissue to gain access to additional chambers. Larval phytophagy is very rare in the Braconidae and has never been confirmed for the subfamily Braconinae (Taylor *et al.* 1996).

Eupelmus (*Macroneura*) *semiputata* (Girault) (Eupelmidae).—*Eupelmus semiputata* was moderately common from galls at Morayfield but rare at Peregian and not collected from Woodburn (Table 1). Of the 103 reared in the study (5.1% of total specimens reared), 74 came from galls collected in 1998. During 1999 only ten *E. semiputata* were reared. We did not encounter any larvae in our dissections. Molecular sequences of adults from Morayfield and Peregian were identical (Table 2). There is only a single described Australian species of *Eupelmus* (*Macroneura*) although Bouček (1988) indicated a second, presumably undescribed, Australian species. The specimens reared in this study appeared to match the holotype of *E. semiputata*.

Species of *Eupelmus* (*Macroneura*) are cosmopolitan, primary or secondary parasites of a wide variety of insect hosts in concealed locations, such as within galls, grass stems, or cocoons. Some species are highly polyphagous, sometimes attacking hosts from several different orders (Gibson 1990). A. A. Girault, in his unpublished manuscript (see Dahms 1978), recorded *E. semiputata* emerging from cecidomyiid galls on Pitted bluegrass, *Bothriochloa decipiens* (Hackel) C. E. Hubbard (as *Andropogon pertusus* (L.) Willdenow). Several other species of chalcidoids were also reared from these galls. CJB has also reared specimens of *E. semiputata* from final instar larvae of *Aspidomorpha deusta* (Fabricius) (Coleoptera: Chrysomelidae), most probably as a hyperparasitoid through an unidentified tachinid. This is the first record of *E. semiputata* emerging from galls of Fergusoninidae.

Chromeurytoma sp. (Pteromalidae).—Specimens of *Chromeurytoma* were reared in low numbers from all three sites (Table 1) and comprised 4.6% of the total specimens reared. Molecular sequences of the D2 gene were obtained only from adults and larvae from Morayfield (Table 2) but,

based on morphology, adults from all three sites appear to be the one species. There are fourteen described species of *Chromeurytoma*, all from Australia. The species reared in our study could not be assigned to one of the described species.

The larvae of *Chromeurytoma* sp. were normally active and conspicuously setose. They were relatively elongate, gradually tapering posteriorly. The body also tapered anteriorly to a relatively small head capsule. The mandibles of mature larvae were thin with a single, strong, acute tooth. The heavily setose bodies of *Chromeurytoma* larvae distinguished them from most other larvae within the galls. *Neanastatus* larvae were superficially similar but less setose, lacking the elongate ventrolateral setae found on the abdominal segments of *Chromeurytoma* larvae. In addition, *Chromeurytoma* larvae had lateral setae on the first abdominal segment (absent in *Neanastatus*) and had the most posterior pair of setae on the head capsule more widely separated. The bases of the posterior setae on the head capsule were separated by more than twice the length of a seta in *Chromeurytoma* larvae, but only by about the length of a seta in *Neanastatus* larvae. *Chromeurytoma* larvae usually had a conspicuous dorsal hump between the first and second abdominal segments and a series of thin, transverse, sclerotized, intersegmental bands between the thoracic and first seven abdominal segments.

Chromeurytoma larvae were most commonly solitary hyperparasitoids through other Hymenoptera within the galls, feeding on their mature larvae or pupae. *Chromeurytoma* larvae or pupae were recovered from chambers containing the remains of *Neanastatus* and *Eurytoma* pupae and from chambers containing moribund *Bracon* larvae or their head capsules. On two occasions, *Chromeurytoma* larvae occupied isolated chambers containing the remains of lightly sclerotized *Fergusonina* larvae, and possibly developed as primary ectoparasitoids of the fly.

Species of *Chromeurytoma* have been reared from unidentified galls on species of *Eucalyptus* and *Acacia* (Bouček 1988). This study is the first to record *Chromeurytoma* emerging from galls of Fergusoninidae and the first to document the larval biology of a member of the genus.

Megastigmus sp. (Torymidae).—Specimens of *Megastigmus* were reared in low numbers from all three sites (Table 1) and comprised 4.0% of the total specimens. Of the 80 adults reared during the entire study, 55 emerged from galls collected in 1997. Molecular sequences of adults from Morayfield and Woodburn were identical (Table 2). *Megastigmus* is a large genus distributed throughout most of the world, except the Neotropics. It is particularly speciose in Australia with 47 described species (Bouček 1988). In the absence of any revisionary studies on the genus and given that species often display considerable variation in size, color and sculpture (Bouček 1988), no attempt was made to identify the species reared in our study.

Larvae of *Megastigmus* sp. were not encountered in our original dissections of galls from which specimens were sequenced. However, examination of larval exuviae recovered from *Fergusonina* puparia from which *Megastigmus* sp. adults had emerged, enabled us to identify larvae of *Megastigmus* sp. in subsequent gall dissections. Larvae resembled those of *Eurytoma* sp., but mature *Megastigmus* larvae could be distinguished from those of *Eurytoma* sp. and all other gall-associated wasps by their distinctive mandibles. Each mandible was 4-dentate, with a large, acute, apical tooth and three small teeth evenly spaced along its inner cutting edge. They closely resemble the larval mandibles of *Megastigmus dorsalis* (Fabricius) figured by Askew (1966).

The larval biology of *Megastigmus* sp. was variable. Most commonly, adults emerged from intact *Fergusonina* puparia found within isolated gall chambers. Each parasitized puparium contained the exu-

vium of the final instar *Megastigmus* larva and meconium in the form of numerous black, discrete pellets. Presumably, *Megastigmus* sp. developed as a primary parasitoid, either as an endoparasitoid or ectoparasitoid of the pharate *Fergusonina* pupa. We found no evidence that *Megastigmus* developed as a hyperparasitoid within puparia. Less commonly, *Megastigmus* appeared to develop as a solitary ectoparasitoid of third instar *Fergusonina*. *Megastigmus* larvae and naked pupae were found within isolated gall chambers that contained pale, granulate host remains including dissociated *Fergusonina* dorsal shield plates. In these cases the voided larval meconium consisted of a thick mass instead of discrete pellets. In addition, *Megastigmus* sp. also developed as a hyperparasitoid through *Bracon* sp. On several occasions, *Megastigmus* larvae, pupae or pharate adults were found within cocoons with the remains of *Bracon* prepupae, pupae or pharate adults.

Megastigmus is a biologically diverse genus with species having larval biologies ranging from obligate plant feeders to obligate parasitoids (Bouček 1988). Currie (1937) reared two species of *Megastigmus*, *M. quinquesetae* (Girault) and an unidentified species, from *Fergusonina nicholsoni* flower-galls on *Eucalyptus macrorhyncha*. He reported that the larvae of both species were inquilines within the galls, forming their own separate chambers and presumably feeding on gall tissue. In contrast, the *Megastigmus* in our study appears to be entirely entomophagous. Taylor *et al.* (1996) also reared two species of *Megastigmus* from *Fergusonina flavicornis* leaf-galls on *E. camaldulensis* but they did not investigate their larval biologies.

Eupelmus (*Eupelmus*) sp. (Eupelmidae).—Specimens of *Eupelmus* were recovered in very low numbers from all three sites and accounted for 0.5% of total specimens reared (Table 1). They appeared to belong to a single species and the molecular sequences of adults from Morayfield and Peregian were identical (Table 2). *Eupelmus* sp. larvae were not sequenced as none were encountered during dissections. A single adult female was found in an isolated gall chamber together with a lightly sclerotized, collapsed *Fergusonina* prepupa. The pupal exuvium of the wasp was also present in the chamber. The precise larval biology of *Eupelmus* sp. is unknown although it is clearly a solitary primary parasitoid or hyperparasitoid. There are many species of *Eupelmus* (*Eupelmus*) found throughout the world; they are parasitic, or rarely 'predatory', on a wide variety of hosts (Bouček 1988). Harris (1982) also reported a species of *Eupelmus* emerging from *Fergusonina syzygii* Harris galls on *Syzygium cumini* (L.) (Myrtaceae) in India.

Euderus sp. (Eulophidae).—Specimens of *Euderus* were recovered in very small numbers from all three sites and accounted for 0.5% of total specimens reared (Table 1). They appeared to represent a single species and the molecular sequences of single adults from Peregian and Woodburn were identical. *Euderus* larvae and pupae were not encountered during dissections. However, the D2 gene sequence of a single egg matched that of the adult *Euderus* (Table 2). The egg was attached to a moribund *Poecilocryptus nigromaculatus* (see below) larva that had been feeding on a *Fergusonina* puparium. Evidently *Euderus* sp. acts a hyperparasitoid within the galls, which might explain its low relative abundance. *Euderus* is a large cosmopolitan genus with species attacking larval Lepidoptera and Coleoptera (Bouček 1988). Species are also known to be hyperparasitic, attacking Braconidae (Bouček 1988). Taylor *et al.* (1996) also reared a species of *Euderus* in low numbers from *Fergusonina flavicornis* leaf-galls. They suggested that its larva might be hyperparasitic on *Bracon fergusonorum* within the galls.

Poecilocryptus nigromaculatus Cameron (Ichneumonidae).—*Poecilocryptus nigroma-*

culatus was the rarest gall-associated wasp species at 0.3% of the total specimens reared, with only one and four specimens recovered from Morayfield and Woodburn respectively (Table 1). The specimens appeared to be *P. nigromaculatus*, although they differed slightly in coloration, lacking the black markings on the second gastral tergite normally found in this species (Gauld and Holloway 1986).

Mature *P. nigromaculatus* larvae were distinguished from most gall-associated wasp larvae, except those of *Bracon* sp., by their large size. They could be distinguished from *Bracon* larvae by their very large, heavily sclerotized, bidentate mandibles and by a large sclerotized plate on the postlabium (see Short 1978).

Only a single *P. nigromaculatus* larva and a single pupa were recovered from dissections of galls from Woodburn, Morayfield and Peregian but several larvae and prepupae were found in additional dissections of galls from Bracken Ridge and Coolum (QLD). Only a single mature larva, prepupa or pupa of *P. nigromaculatus* was observed per gall. Each gall had its internal structure highly modified, with most chambers breached and interconnected by relatively large holes. The chambers were generally packed with brown, particulate debris that we concluded was masticated gall tissue. However, various chambers also contained dissociated *Fergusonina* dorsal-shield plates, empty *Fergusonina* puparia rent with large, ragged holes, the remains of *Bracon* pupae and pharate adults, and *Bracon* larval mandibles. *Poecilocryptus nigromaculatus* pupated within the gall in a relatively large central cavity, incorporating several chambers, presumably excavated by the larva. Pupation occured inside a brown, moderately densely woven cocoon with brown debris incorporated on its outer surface.

Poecilocryptus (Subfamily Labeninae) is an endemic Australian genus associated with a variety of galls on trees of the genera *Eucalyptus*, *Acacia*, *Banksia* (Gauld and Holloway 1986, Taylor *et al.* 1996) and now *Melaleuca*. *Poecilocryptus nigromaculatus* has been reared from anthribid weevil galls on *Acacia floribunda* (Ventenat) Willdenow (= *A. longifolia*), eriococcid galls on *Eucalyptus* (Gauld and Holloway 1986), and pteromalid galls on *Acacia* (Noble 1941) including those of *Trichilogaster acaciaelongifoliae* (Froggatt) on *A. floribunda*, and *Perilampella hecataeus* (Walker) on *A. decurrens* Willdenow. *Poecilocryptus nigromaculatus* has also been recorded from galls of Fergusoninidae, Taylor *et al.* (1996) rearing it and *P. galliphagus* Gauld and Holloway from *Fergusonina flavicornis* Malloch galls on *Eucalyptus camaldulensis*.

There are conflicting reports about the biology of *Poecilocryptus*. We concluded that *Poecilocryptus nigromaculatus* larvae fed on many hosts within each *Fergusonina* gall, using their large mandibles to chew through gall tissue and enter multiple chambers. They appeared to consume the inhabitants of each chamber regardless of its identity. Noble (1941) considered that *P. nigromaculatus* was parasitic upon the larvae of a moth that lived as an inquiline within the multichambered galls of *Trichilogaster acaciaelongifoliae*. He had no direct evidence of this host association but specifically noted an adult *P. nigromaculatus* occupying a gall that had much of its interior hollowed out by what he assumed was a moth larva. However, his description bears a striking similarity to the situation we observed in the *Fergusonina* galls and we suggest that *P. nigromaculatus* is probably also a generalist parasitoid within *Trichilogaster* galls.

Members of the tribe Poecilocryptini all appear to oviposit within nutritious plant tissue (Gauld and Holloway 1986). The enormous mandibles of their larvae led Short (1978) to speculate that they may be, at least in part, phytophagous. According to Quicke (1997) partial phytophagy has been confirmed for a species of *Poecilocryptus* living within coccoid-induced

Table 3. Summary of the biologies of Hymenoptera associated with galls of *Fergusonina* sp. *Eupelmus semiputata* not included due to lack of information. ? = Some evidence but not confirmed.

Species	Primary Endoparasitoid	Primary Ectoparasitoid	Hyperparasitoid	Single hosts	Multiple hosts
Eurytoma sp.	?	X		X	X
Coelocyba sp.		X		X	
Neanastatus sp.		X		X	
Cirrospilus sp.		X		X	
Bracon sp.		X	X	rarely	X
Chromeurytoma sp.		?	X	X	
Megastigmus sp.	?	X	X	X	
Eupelmus (*Eupelmus*) sp.				X	
Euderus sp.			X		
Poecilocryptus nigromaculatus		X	X		X

galls. We confirmed that *Poecilocryptus* larvae masticate a considerable amount of *Melaleuca* gall tissue, evidenced by copious amounts of brown debris. However, it is unclear whether they derived nutrition from the plant tissue or just mechanically eroded the chamber walls to gain access to their contents.

CONCLUSION

Of the eleven species of Hymenoptera associated with the *Fergusonina* sp. gall, we were able to observe and describe the biology of nine of the species (Table 3). Seven commonly or exclusively fed directly upon the *Fergusonina* larvae and or pupae with most species developing on a single host. However, three of the seven usually fed on multiple hosts. These species have specialized biologies, which enable them to chew through plant tissues to access gall inhabitants. On the available evidence, the remaining two species appeared to be hyperparasitoids. We do not know which of the eleven species are gall specific, but did find it interesting that none of these species were reared from other galls on *M. quinquenervia* formed by Cecidomyiidae or Homoptera (unpublished data). None of the Hymenoptera in this study could be described as inquilines. The term inquiline is defined by Torre-Bueno (1989) as a commensal that lives in a very close spatial relationship with its host, in its shelter, not feeding on it, but frequently destroying it. We did occasionally observe species of Lepidoptera acting as inquilines, including one species, *Holocola* sp. (Tortricidae), which is known to feed on young leaf and flower buds of *M. quinquenervia* (unpublished data).

We found the D2 molecular method to be robust for characterizing all life stages including eggs and small larvae. Further, the D2 gene sequence was consistent for each species and between adults and immatures. In the dissections we encountered the immature forms of eight out of eleven species, which were matched with the adult forms. The molecular technology provides many advantages in the study of cryptic immature insects. The amount of time and effort required to identify immatures is greatly reduced because rearing to the adult stage is not needed. The biology of the immatures can be observed *in vivo* and matched with adults without speculation or comparison to known biologies of related species. A greater number of gall-inhabiting insects are likely to be discovered using this technique as compared to other techniques. Removal of the gall from the plant and holding it for insect emergence subjects the inhabitants to changes in plant turgor, humidity and temperature. All of these factors could be critical to the survival of the immatures. Sleeving the gall for collection of emerging

insects may be biased against late arrivals such as hyperparasitoids.

Gene sequences are quantitative and can be compared to sequences collected later in the study or by other researchers. By posting the sequence on GenBank® other researchers may in turn match the identity of their insects. Sequence data serves as an interim identity for the insect species until they are described. Field studies and biological control programs in particular should submit vouchers not only of the insect specimens but also of the gene sequences as well. Later revisions of genera could include, where possible, the molecular data from a wide array of biological studies. In this way a greater number of specimens could be identified simultaneously. Our understanding of the biology and distribution of insect species would be greatly enhanced.

In biological control programs directed against weeds, agents must reach high population levels in order to control their host. Development of high population levels in the region of introduction is promoted initially by an almost unlimited food supply and by release from the agent's natural enemies (Harley and Forno 1992). *Fergusonina* sp. is likely to be introduced to Florida, USA, where it will find an abundance of suitable *M. quinquenervia* plant buds which it needs to form galls (Goolsby *et al.* 2000a, Van *et al.* 2000). In its region of origin *Fergusonina* sp. is heavily attacked by natural enemies, including eight primary parasitoids. One would predict that fewer parasitoid species would attack *Fergusonina* sp. in Florida, and that they would be less co-adapted than those in Australia. Fergusoninidae are not represented in the New World, so the association with this family of gall-making flies would be novel for the indigenous parasitoids. In the absence of its co-evolved natural enemies, *Fergusonina* sp. could reach much higher populations levels, potentially having an impact on *M. quinquenervia*. We hope that our study provides the basis for future comparisons of natural enemies of *Fergusonina* sp. in both its native and adventitious range. This research would further our ability to predict the impact of indigenous parasitoids on introduced biological control agents.

ACKNOWLEDGEMENTS

The authors wish to thank John LaSalle, Mike Rose, and the referees for their helpful comments in preparation of the manuscript; Diana Hartley and Leslie McKenzie for molecular sequencing; Matthew Purcell, Ted Center and Gary Buckingham for collaborative insights; Jeff Wright for photographs of the galls. Research funding was provided by South Florida Water Management District, Army Corps of Engineers, Florida Department of the Environment, and USDA-ARS-Office of International Research Programs.

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